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A strong quantitative trait locus for wing length on chromosome 2 in a wild population of great reed warblers

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Wing length is a key character for essential behaviours related to bird flight such as migration and foraging. In the present study, we initiate the search for the genes underlying wing length in birds by studying a long-distance migrant, the great reed warbler (*Acrocephalus arundinaceus*). In this species wing length is an evolutionary interesting trait with pronounced latitudinal gradient and sex-specific selection regimes in local populations. We performed a quantitative trait locus (QTL) scan for wing length in great reed warblers using phenotypic, genotypic, pedigree and linkage map data from our long-term study population in Sweden. We applied the linkage analysis mapping method implemented in GridQTL (a new web-based software) and detected a genome-wide significant QTL for wing length on chromosome 2, to our knowledge, the first detected QTL in wild birds. The QTL extended over 25 cM and accounted for a substantial part (37%) of the phenotypic variance of the trait. A genome scan for tarsus length (a body-size-related trait) did not show any signal, implying that the wing-length QTL on chromosome 2 was not associated with body size. Our results provide a first important step into understanding the genetic architecture of avian wing length, and give opportunities to study the evolutionary dynamics of wing length at the locus level.

Keywords: quantitative trait locus; genome scan; genetic architecture; pedigree; linkage map; wing length

1. INTRODUCTION

The inheritance, genetics and evolution of continuously varying, quantitative traits have been of constantly growing interest ever since Darwin proposed the idea of natural selection as a process forming the diversity of life (Darwin 1859). A main question yet to be fully understood is how the genetic variation of traits under strong directional selection is being maintained. Fisher's fundamental theorem states that the rate of change in fitness equals the amount of additive genetic variance in fitness itself (Fisher 1930). As a consequence, fitness-related traits are expected to have low heritability (Fisher 1930; Edwards 2000). This pattern is consistent with empirical heritability estimates (Mousseau & Roff 1987); however, when dissecting the variance components, fitness-related traits have unexpectedly high additive genetic variance. Therefore, it has been suggested that the low heritability of fitness-related traits is mainly a consequence of higher environmental variance (Merilä & Sheldon 2000), contradicting the rationale behind Fisher's theorem. Recent advancements in molecular biology and bioinformatics now allow the exploration of the underlying genetic architecture of quantitative traits in natural populations, and these advancements also promise important insights into the mechanics of selection, adaptation, microevolution and how variation is maintained in traits under selection (Ellegren & Sheldon 2008). Quantitative trait locus (QTL) mapping can be used to detect regions in a genome that are associated with a certain trait, and is the first step towards a detailed understanding of the genetic architecture of ecologically and evolutionary interesting complex traits (Lynch & Walsh 1998; Erickson *et al*. 2004; Slate 2005).

Many successful QTL studies conducted in laboratory populations have contributed importantly to evolutionary biology, for example, with insights into the genetic architecture of quantitative traits, epistatic and pleiotropic effects and genotype by environment interactions (Mackay 2001; Carlborg & Haley 2004; Flint & Mackay 2009). However, there is a risk that the mapping results from laboratory-based studies do not mirror the natural situation because the laboratory environment is kept constant, the genetic variance of the trait is deliberately affected by the crossing scheme, and long-time exposure to laboratory conditions may unintentionally affect the genetic variance owing to genetic drift and adaptation (Hoffmann 2002). In wild populations, on the other hand, the environmental variance of quantitative traits is often high and the genetic variance can be preserved by shifting selection regimes and large population sizes

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(Falconer & Mackay 1996). This suggests that QTL studies of natural populations will provide additional and novel insights into the genetics of quantitative traits compared with studies based on data from the laboratory. Indeed, this has been acknowledged in experimental studies of QTL effects under a range of natural environments in various plant species, such as different crops and *Arabidopsis thaliana* (Koornneef *et al.* 2004; Mitchell-Olds *et al.* 2008; Verhoeven *et al.* 2008; Alonso-Blanco *et al.* 2009), and to some extent also in animals, including in pea aphids (*Acyrthosiphon pisum*; Hawthorne & Via 2001), crickets (*Laupala* spp.; Shaw *et al.* 2007) and zebra fish (*Danio rerio*; Wright *et al.* 2006). QTL mapping in completely unmanipulated natural populations is however still in its infancy, initiated by Slate *et al.*'s QTL scan on birth weight in red deer (*Cervus elaphus*; Slate *et al.* 2002). Since then, genome screenings for both Mendelian (coat colour, horn type) and quantitative traits (parasite resistance, birth date, birth weight, horn length and different body-size-related morphological traits) have been performed in Soay sheep (*Ovis aries*; Beraldi *et al.* 2006, 2007*a*,*b*; Johnston *et al.* 2010). A reason for why QTL studies in wild populations are still scarce is because of the difficulties involved in collecting long-term, multi-generational phenotypic and genotypic data in non-model organisms in their natural environments.

The great reed warbler (*Acrocephalus arundinaceus*) is a socially polygynous long-distance migrant that spends the winter in tropical Africa and breeds in Europe and western Asia (Cramp 1992). This species has expanded its range northwards post-glacially and colonized Sweden as recently as the late 1950s (Hansson *et al.* 2000, 2008). The recent northward expansion with novel migratory routes and habitats has most probably introduced new selection regimes in the species. One trait that could be particularly affected by these selection pressures is wing length, a key character for essential behaviours related to bird flight such as foraging and migration (Savile 1956; Alatalo *et al.* 1984*b*; Mulvihill & Chandler 1990). In line with this reasoning, there is substantial variation in wing length over the Eurasian breeding range of the great reed warbler, with southern populations having shorter wings (mean male wing length $\pm$ s.e. (mm); Turkey: 95.6 ± 0.60, O. Kesapli Didrickson 2009, personal communication; Portugal: 97.4 ± 0.37, J. M. Neto 2009, personal communication), central populations intermediate wings (Czech Republic: 99.2 ± 0.32, P. Prochazka 2009, personal communication; Ukraine: 98.7 ± 0.45, A. Poluda 2000, personal communication) and northern populations longer wings (Latvia: 101.3 ± 0.40, S. Bensch 1992, unpublished data; Sweden: 101.3 ± 0.12, Åkesson *et al.* 2007*b*). Because all these populations winter in tropical Africa, and hence have gradually increasing distances to their wintering sites when breeding further north in Europe, these data suggest that selection on wing length is related to migration distance on a wider geographical scale in great reed warblers.

We have studied the dynamics of wing length in great reed warblers in our long-term study population in southern Central Sweden, at the northwestern-most range limit of the species' distribution (Bensch 1996; Hasselquist 1998; Bensch *et al.* 2007). We know from previous analyses that wing length is under selection in our study population, and that the selection regimes differ between the sexes, with females experiencing negative and males positive directional selection (Åkesson *et al.* 2007*b*; Tarka *et al.* in preparation). Great reed warblers inhabit dense reeds, which is a habitat that requires excellent manoeuvrability skills during foraging and when feeding nestlings, this is of particular importance for females that often are mated to polygynous males and have to feed nestlings alone (Bensch 1996; Sejberg *et al.* 2000). On the other hand, rapid spring migration with early arrival at the breeding grounds, achievement of a high-quality territory and early onset of breeding are highly important for reproductive success in these long-distance migrating warblers, especially so in males aiming at attracting several mates (Hasselquist 1998). Thus, it is likely that there is a trade-off within local great reed warbler populations between a longer wing that is optimized for migratory flights and a shorter wing optimized for manoeuvrability during breeding. Different selection pressures over the distribution range, during different life stages, both between and within males and females, could maintain the genetic variation in wing length.

Dissecting the genetic architecture of wing length in migratory birds is a necessary step to obtain complete understanding of the evolutionary dynamics of avian flight. In the present study, we performed a QTL scan for wing length in great reed warblers by using phenotypic, genotypic, pedigree and linkage map data collected during 21 years in our long-term study population (Hansson *et al.* 2005; Åkesson *et al.* 2007*a*; Dawson *et al.* 2007). To evaluate whether putative QTLs are associated explicitly with wing length, or alternatively related more generally with body size, we also performed a screen for tarsus length. Both these traits are typical quantitative traits that can be expected to be controlled by many genes, each with a small effect (Mackay *et al.* 2009).

2. MATERIAL AND METHODS

(a) *Study population*

The great reed warbler (*A. arundinaceus*) is a large Sylviidae warbler with a wide breeding range in Eurasia (Cramp 1992; Helbig & Seibold 1999). It has a socially polygynous mating system where males can have up to five females during a breeding season (Hasselquist 1998). The sexes are phenotypically similar, with males being slightly larger than females. The species is a long-distance migrant that winters in sub-Saharan Africa and breeds in reed lakes in Europe and western Asia. Our long-term study population at Lake Kvismaren in southern Central Sweden (50°10′ N, 15°25′ E) is located in the northernmost part of the species' breeding range. It was founded by a few individuals in 1978 and has been studied since 1983 (e.g. Bensch 1996; Hasselquist 1998; Bensch *et al.* 2007). The size of the population increased to 50–60 individuals during the 1980s, but has decreased to *ca* 40 during the last years of this study. The species colonized Sweden in the 1950s and the total Swedish population now numbers around 900 individuals (Hansson *et al.* 2002). Our study population is semi-isolated and therefore affected by the immigration and emigration dynamics within Sweden and possibly to some extent also with the rest of northern Europe (Hansson *et al.* 2002, 2003). Most adults that survive the winter return to breed

in the same lake in consecutive years (*ca* 95%), while among nestlings that survive until breeding age, approximately half return to their natal site, whereas the other half disperse to nearby breeding sites (Bensch *et al.* 1998; Hansson *et al.* 2002). In our population, individuals are ringed with unique colour ring combinations and the breeding success is monitored daily over the season. Almost all birds (95–100%) are captured each year, measured for several morphological characters and blood sampled (Bensch *et al.* 1998).

(b) *Phenotypes and pedigree*

The pedigree included 878 individuals breeding between 1983 and 2004, of which 133 were immigrants with unknown parents. Parentage is easily assigned in the field and the level of extra-pair paternity is low (*ca* 3% of the nestlings are extra-pair young; Hasselquist *et al.* 1996; Arlt *et al.* 2004; Hansson *et al.* 2004*a*). All cases of miss-assigned parentage in the pedigree were corrected using genotyping data (cf. Hansson *et al.* 2005).

In this study, we focus on wing length, a trait that influences flight performance and is hence a crucial trait for a long-distance migrant bird. We also included tarsus length in our analyses in order to evaluate whether a putative QTL was associated explicitly with wing length, or alternatively connected more generally with body size. Tarsus length is often used as a proxy for overall body size measurement in song birds (e.g. Kruuk *et al.* 2001). In great reed warblers, wing length is both highly heritable (additive genetic variance (V_A) = 2.21 ± 0.36 s.e., heritability (h^2) = 0.67 ± 0.08 s.e.) and under selection, while tarsus length with a similar heritability ($V_A = 0.46 \pm 0.07$ s.e., $h^2 = 0.71 \pm 0.08$ s.e.) is not subject to any detectable selection pressure (Åkesson *et al.* 2007*b*, 2008). The quantitative genetic parameters were similar in the present dataset (wing length: $V_A = 2.18$, $h^2 = 0.68$; tarsus length: $V_A = 0.37$, $h^2 = 0.60$; table 1; for methods, see Åkesson *et al.* 2008). The traits are not genetically correlated (Åkesson *et al.* 2007*b*, 2008), which was also confirmed in the present dataset (0.047 ± 1.14 s.e.). Both traits were measured on adult birds, wing length according to method 3 in Svensson (1995) and tarsus length according to Alatalo *et al.* (1984*a*). In total, 333 unique individuals were measured for wing length and tarsus length. Of these, 170 (wing length) and 154 (tarsus length) individuals were measured more than once for each trait (repeated measures were taken into account in the model; §2*d*). Non-phenotyped birds were included in the calculation of the identity-by-descent matrices to improve the estimation of the identity-by-descent probabilities.

We used mixed models in SAS 9.2 software (SAS Institute Inc., Cary, NC, USA) to find significant fixed factors that consistently affect the mean of the trait but are not in focus in the present study. Wing length was corrected for sex (males have consistently longer wings than females) and for age (the wing grows longer after moulting during the first 2 years and reaches a plateau when the bird reaches the age of 3 years; Hasselquist *et al.* 1996). Tarsus length was corrected for sex (males have longer tarsus than females) and for ringer (differences between measuring personnel).

(c) *The linkage map*

We used the great reed warbler linkage map described in Hansson *et al.* (2005) and Åkesson *et al.* (2007*a*). We ran analyses using the framework map (i.e. the map that includes only markers with a single statistically significant map position) and the parsimonious map (i.e. the best-order map that includes also less informative markers with more than one statistically possible position). The parsimonious map has an advantage of a wider genome coverage than the framework map, but the uncertain order of some markers may affect the analyses. Because marker variability has a strong impact on the statistical power in a QTL scan (Slate *et al.* 1999), we excluded all markers with less than 100 informative meioses from all analyses. There is a pronounced sex-biased recombination in the great reed warbler, where the female recombination rate is approximately twice as high as in males (Hansson *et al.* 2005). In this study, we used sex-average linkage map distances.

The framework map that we used in our QTL analyses consisted of 34 autosomal and four Z-linked microsatellite markers, and 11 autosomal and two Z-linked amplified fragment length polymorphism (AFLP) markers, distributed over 12 autosomal linkage groups and the Z group (corresponding to the following chicken chromosomes: LG1/Gga3; 2/19; 3/2; 4/24; 5/1; 6/5; 7/4; 12/21; 13/1; 15/—; 18/—; 20/—; Z/Z). The framework map had a total length of 388.7 cM with a density of 7.6 cM per marker and a coverage of approximately 20 per cent of the genome. The parsimonious map consisted of 50 autosomal and seven Z-linked microsatellite markers, and 31 autosomal and five Z-linked AFLP markers, distributed over 16 autosomal linkage groups and the Z group (the linkage groups corresponded to the following chicken chromosome: LG1/Gga3; 2/19; 3/2; 4/24; 5/1; 6/5; 7/4; 8/8; 9/13; 10/9; 11/7; 12/21; 13/1; 15/—; 18/—; 20/—; Z/Z; Åkesson *et al.* 2007*a*; Dawson *et al.* 2007). The total map length was 698.4 cM, with an average density of 7.5 cM per marker and a coverage of approximately 40 per cent of the genome (Åkesson *et al.* 2007*a*; Dawson *et al.* 2007).

(d) *QTL analyses*

We used the newly developed web-based software GridQTL (www.gridqtl.org.uk) to perform a genome-wide scan for QTLs (Hernández-Sánchez *et al.* 2009). The software implements association mapping (linkage disequilibrium (LD) mapping) and linkage analysis (LA) (Pong-Wong *et al.* 2001; Meuwissen *et al.* 2002). LA is based on co-segregation between markers and a QTL in the pedigree, and is more robust to false positives than LD. LA is highly suitable for low-resolution scans using low-density linkage maps as in our study. LD mapping is based on the association between marker and trait loci on the population level, i.e. independent of the co-segregation in the pedigree, and provides a high mapping resolution when high-density maps are available. Because our linkage map has a low marker density, we did not use the LD-mapping option.

George *et al.* (2000) suggested a two-step variance component analysis that is the basis for most QTL applications, and this method is implemented in GridQTL (Hernández-Sánchez *et al.* 2009). It compares a polygenic model (many genes with a low effect evenly spread out in the genome)

$$y = X\beta + Za + Zpe + e,$$

with a QTL model (few genes with a large effect)

$$y = X\beta + Za + Zpe + Zq + e,$$

where y is a vector of phenotypes, β is a vector of fixed effects, a a vector of additive polygenic effects, pe a vector of permanent environment effects, e a residual vector and

Table 1. Trait descriptions with estimated variance components.

							additive genetic (heritability)		QTL		permanent environment		residual	
trait	phen. n[a]	mean[b]	V_{OBS}[c]	V_P[d]	map	model	V_A[e]	h^{2}[f]	V_Q[g]	q^{2}[h]	V_{PE}[i]	e^{2}[j]	V_E[k]	ε^{2}[l]
wing length	658 [333]	98.3 (1.7)	7.98	3.19 (0.28)	parsimonious	polygenic	2.18 (0.39)	0.68 (0.08)			0.39 (0.21)	0.12 (0.07)	0.63 (0.05)	0.20 (0.02)
				3.21 (0.28)		QTL	1.02 (0.47)	0.32 (0.14)	1.15 (0.40)	0.36 (0.11)	0.41 (0.20)	0.13 (0.07)	0.63 (0.05)	0.20 (0.02)
				3.19 (0.28)	framework	polygenic	2.18 (0.39)	0.68 (0.08)			0.39 (0.21)	0.12 (0.07)	0.63 (0.05)	0.20 (0.02)
				3.20 (0.28)		QTL	0.95 (0.46)	0.30 (0.14)	1.18 (0.39)	0.37 (0.11)	0.43 (0.20)	0.13 (0.07)	0.63 (0.05)	0.20 (0.02)
tarsus length	597 [333]	33.2 (1.0)	0.98	0.61 (0.05)	parsimonious	polygenic	0.37 (0.08)	0.60 (0.09)			0.18 (0.05)	0.30 (0.09)	0.06 (0.005)	0.09 (0.01)
				0.61 (0.05)		QTL	0.37 (0.08)	0.60 (0.09)	0 (0)	0 (0)	0.18 (0.05)	0.30 (0.09)	0.06 (0.005)	0.09 (0.01)
				0.61 (0.05)	framework	polygenic	0.37 (0.08)	0.60 (0.09)			0.18 (0.05)	0.30 (0.09)	0.06 (0.005)	0.09 (0.01)
				0.61 (0.05)		QTL	0.37 (0.08)	0.60 (0.09)	0 (0)	0 (0)	0.18 (0.05)	0.30 (0.09)	0.06 (0.005)	0.09 (0.01)

[a]Number of phenotyped individuals. Within square brackets: number of uniquely phenotyped individuals.
[b]Trait mean (mm) with s.e. within brackets.
[c]Observed phenotypic variance.
[d]Estimated phenotypic variance, obtained by summing the variance components with s.e. within brackets.
[e]Additive genetic variance with s.e. within brackets.
[f]Heritability with s.e. within brackets.
[g]QTL (quantitative trait locus) variance with s.e. within brackets.
[h]QTL effect with s.e. within brackets.
[i]Permanent environmental variance with s.e. within brackets.
[j]Permanent environmental effect with s.e. within brackets.
[k]Residual variance with s.e. within brackets.
[l]Residual effect with s.e. within brackets.

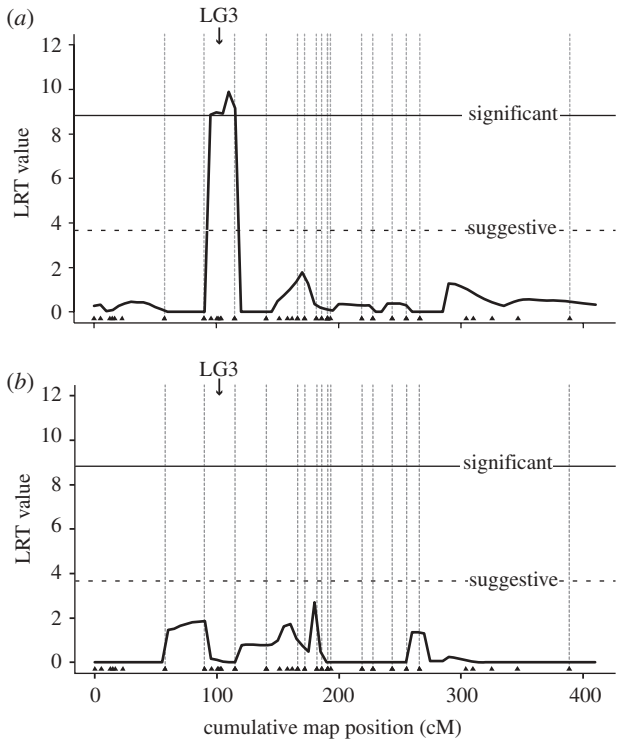

Figure 1. Genome-wide QTL scan every 5 cM for (*a*) wing length and (*b*) tarsus length in the great reed warbler using the framework linkage map. LRT values were plotted against genetic position (cM). Genome-wide significant and suggestive thresholds are indicated by horizontal lines. Vertical lines mark the linkage group boundaries and linkage group 3 is highlighted (LG3).

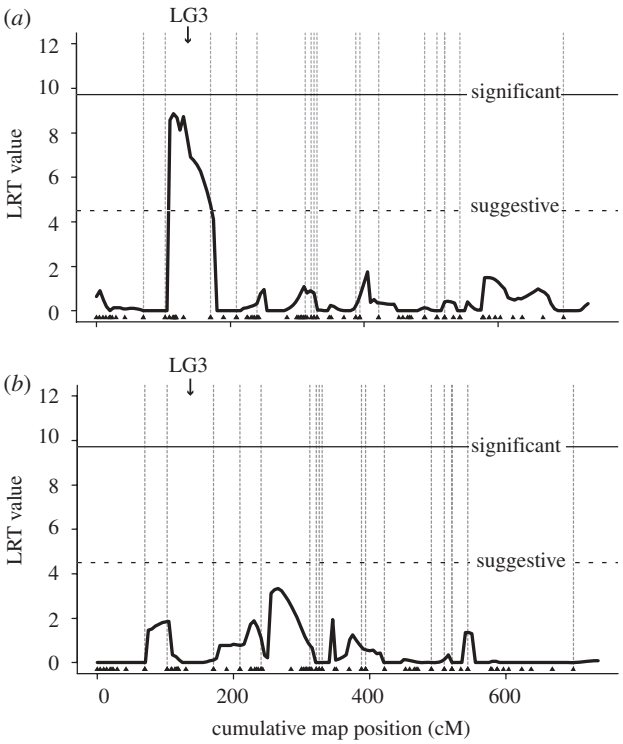

Figure 2. Genome-wide QTL scan every 5 cM for (*a*) wing length and (*b*) tarsus length in the great reed warbler using the parsimonious linkage map. LRT values were plotted against genetic position (cM). Genome-wide significant and suggestive thresholds are indicated by horizontal lines. Vertical lines mark the linkage group boundaries and linkage group 3 is highlighted (LG3).

X and Z are design matrices that relate records to fixed and random effects, respectively. In the QTL model, q is a vector of additive QTL effects. The significance of the QTL effect was assessed by a likelihood ratio test (LRT), using the test statistic:

$$\mathrm{LRT} = -2\ln(L_{\mathrm{polygenic}}/L_{\mathrm{QTL}}),$$

where $L_{\mathrm{polygenic}}$ is the likelihood estimate of the polygenic model, and L_{QTL} for the QTL model.

In the models, we included individual identity as a random factor to account for permanent environment effects such as non-genetic between-individual variance and to correct for repeated measures. We also tested for common environment effects such as maternal and year effects, but since these effects were not statistically significant and did not affect the additive genetic or QTL variance, they were excluded from the model. As mentioned above, we also included fixed factors (sex and age for wing length; sex and ringer for tarsus length). We set T (i.e. the number of generations between population founders and pedigree founders) to zero in the history file, which excludes the LD-mapping option and makes the program run an LA analysis exclusively (see LDLA user manual, v. 0.4-10/11/2008; J.-A. Grunchec, J. Hernández-Sánchez & S. Knott 2009, unpublished data). The complete genome scan was performed testing for a QTL every 5 cM for both traits (wing and tarsus length) and using both linkage maps (parsimonious and framework map). Regions of particular interest were run every 1 cM.

To statistically test the significance of a putative QTL, a threshold for the LRT has to be set. Lander & Kruglyak (1995) and Nyholt (2002) provided a formula that gives two threshold values, one 'suggestive' (signals exceeding a threshold expected to be observed once by chance in a genome scan) and one 'significant' (signals exceeding a threshold expected to be observed by chance in only 5% of the genome scans). These thresholds are valid for an infinitely dense linkage map, and therefore corrections were made to a map density of 10 cM by decreasing them by 20 per cent (Lander & Kruglyak 1995; Nyholt 2002; Slate *et al.* 2002). Thresholds calculated accordingly have also been used in previous wild animal QTL mapping studies (Slate *et al.* 2002; see also Beraldi *et al.* 2007*b*). For a genome-wide threshold for the parsimonious map (698.4 cM and 17 linkage groups), we calculated corrected values of 4.50 for the suggestive threshold and 9.72 for the significant threshold. For the framework map (388.7 cM and 13 linkage groups), the corrected values were 3.66 for suggestive and 8.83 for significant thresholds. For linkage groups of interest, we also calculated chromosome-wide thresholds. For linkage group 3, the corrected chromosome-wide significance threshold was 4.23 for the framework map and 5.49 for the parsimonious map.

3. RESULTS

The results from the wing-length QTL scan using the framework map (figure 1*a*) and the parsimonious map (figure 2*a*) were highly consistent. Over the whole genome, there was one chromosomal region—on linkage group 3 (i.e. chromosome 2)—that was particularly strongly associated with wing length (figures 1*a* and 2*a*). The peak was wide, covering approximately half of

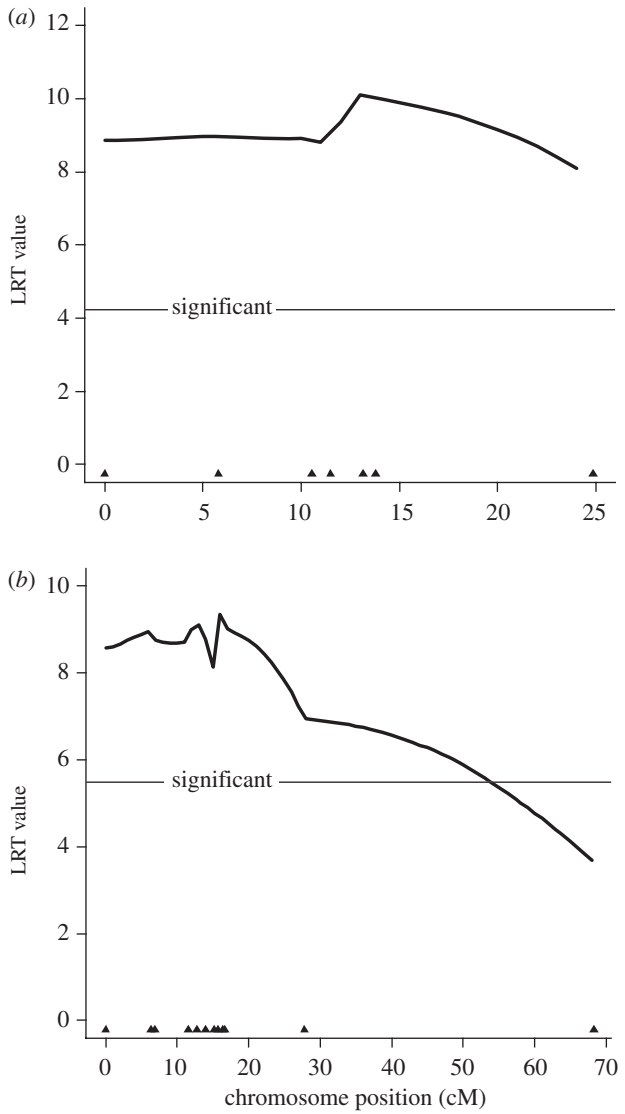

Figure 3. Chromosome-wide QTL scan (every 1 cM) for wing length on linkage group 3 in the great reed warbler, using (*a*) framework and (*b*) parsimonious linkage map. LRT values were plotted against chromosome position (cM). Chromosome-wide significant thresholds are indicated by horizontal lines.

the entire linkage group, stretching over about 25 cM, and included seven markers in the framework map and 10 markers in the parsimonious map. In the framework map, the highest point of the peak was at 13 cM from the start of linkage group 3 (figures 1*a* and 3*a*). The LRT reached 10.12, exceeding the significant genome-wide threshold of 8.83 (figure 1*a*) and the significant chromosome-wide threshold of 4.23 (figure 3*a*). The parsimonious map had the highest peak of 16 cM from the start of linkage group 3 (figures 2*a* and 3*b*). The LRT reached a value of 9.34, exceeding the significant chromosome-wide threshold of 5.49 (figure 3*b*), and not being far from the genome-wide threshold of 9.72 (figure 2*a*). The framework linkage map and the parsimonious map gave analogous results, which indicate that the ambiguous positions of some markers on the parsimonious map did not affect the power of the analyses to a high extent.

For tarsus length, we did not find any suggestive or significant QTL on any linkage group (figures 1*b* and 2*b*). Hence, there were no signs of increased signals on linkage group 3 where the peak for wing length was located.

The variance components from both models are presented in table 1. The QTL explained 37 per cent (framework) and 36 per cent (parsimonious) of the phenotypic variance. When comparing the variance distribution in the polygenic and the QTL model, 53–54% of the additive genetic variance from the polygenic model corresponds to the QTL variance in the QTL model at the highest peak position (13 cM in framework and 16 cM in parsimonious map).

4. DISCUSSION

We have found a significant wing-length QTL at linkage group 3 (i.e. avian chromosome 2) in the great reed warbler. The genome scan for tarsus length did not show any signal in the same region, which implies that the QTL is not directly coupled to body size. Thus, by conducting novel QTL mapping analyses in a wild bird population, we have detected, to our knowledge, the first wing-length QTL in vertebrates.

Phenotypically, wing length is a typical quantitative trait that would be expected to be controlled by many genes spread throughout the genome (Mackay *et al.* 2009). Studies of the genetic architecture of wing morphology in *Drosophila melanogaster* suggest a large number of candidate QTLs (Zimmerman *et al.* 2000; Mezey *et al.* 2005), some of intermediate to large effect (Palsson & Gibson 2004). By contrast, we found a QTL of a very large effect, accounting for more than one-third (37%) of the phenotypic variance in wing length in our great reed warbler population. It is well known in the animal breeding literature that there is a risk for an upward bias of the effect size when estimating the magnitude of a QTL; the 'Beavis effect' (Beavis 1994, 1998). However, this is mainly a problem for studies based on small sample sizes ($n \leq 100$), and becomes negligible for sample sizes exceeding 500. Our pedigree is typical for a free living population: complex, many small families with few offspring scored for adult traits, and the number of phenotyped individuals is close to the lower limit for achieving enough statistical power (Erickson *et al.* 2004). Hence, it is possible that our estimates, being based on a dataset of *ca* 300 phenotyped individuals and a pedigree of *ca* 800 individuals, may be slightly overestimated. Nevertheless, even though the exact magnitude of the wing-length QTL has to be interpreted with some caution, the effect size appears to be very large. Similar strong effects have been found in other study systems. For example, Johnston *et al.* (2010) recently showed that a major QTL for horn length in the Soay sheep population at St Kilda explained all of that trait's additive genetic variance; and Saether *et al.* (2007) concluded (using non-QTL approaches) that the Z chromosome holds one or more genes highly associated with species recognition and reproductive isolation in hybridizing flycatchers (*Ficedula hypoleuca* and *Ficedula albicollis*).

Laboratory studies over the last two decades have shown that adding more markers to a QTL study often results in large QTLs splitting into several closely linked QTLs with a lower average effect size (Mackay *et al.* 2009). This may explain why the effect size decreased somewhat when we used the parsimonious map with many markers, although this could also be owing to the impact of adding markers with ambiguous map position.

Our QTL stretches over a large part (*ca* 25 cM) of linkage group 3 and this region could harbour several wing length genes. As a comparison, the homologous region on chromosome 2 in the chicken (i.e. between *ca* 25 and 125 Mbp; International Chicken Genome Sequencing Consortium 2004; Dawson *et al.* 2007) holds more than 20 QTLs for body-size-related traits (Abasht *et al.* 2006; http://www.animalgenome.org/QTLdb/chicken.html). Moreover, QTLs for body size in the chicken are distributed throughout the chicken genome and are thus not unique to chromosome 2. Unfortunately, no wing characteristics are mapped in chickens, and because our QTL seems unrelated to body size, there are no relevant candidate genes available in the chicken literature (Abasht *et al.* 2006; http://www.animalgenome.org/QTLdb/chicken.html).

The knowledge about the genetic architecture of quantitative traits in wild populations is limited, yet highly important for the understanding of the evolutionary dynamics of such traits. If the main QTL found in this study comprises a single or several tightly linked genes in linkage disequilibrium, the QTL will segregate as an entity, which in turn can facilitate an evolutionary response on standing genetic variation. Indeed, the level of linkage disequilibrium in the study population is relatively high (Hansson *et al.* 2004*b*; B. Hansson & K. Scilléry 2005, unpublished data), probably owing to a recent population bottleneck followed by a rapid expansion in Sweden (Hansson *et al.* 2000, 2002, 2008). To further dissect and fully understand the current QTL, we need to increase the mapping resolution within chromosome 2 and search for possible additional wing-length QTLs in other parts of the genome of the great reed warbler. Our parsimonious linkage map covers approximately 40 per cent of the entire genome, and it is of course possible that there are other QTLs in regions not covered by our map. We will also need to continue to add more phenotyped individuals to the pedigree. This will allow full use of the mapping methods implemented in GridQTL by combining association mapping and LA (Meuwissen *et al.* 2002; Hernández-Sánchez *et al.* 2009), and in this way try to narrow down the QTL region. The relatively high degree of linkage disequilibrium in our study population has mixed consequences; it may have facilitated our search for wing-length QTLs over short and intermediate distances, but on the other hand, it will put constraints on fine-scale mapping.

More than half of the additive genetic variance (53%) was explained by the detected QTL. This means that if selection imposes an evolutionary response on wing length, it is highly likely (probability of 0.53 if we assume only additive effects) that the QTL region on chromosome 2 is involved. However, genetic correlations with other characters would pose evolutionary constraints on wing length. Strong genetic correlations suggest that traits are controlled by the same genes (pleiotropy) or by genes in linkage disequilibrium. Wing and tarsus length in great reed warblers are not genetically correlated (Åkesson *et al.* 2007*b*, 2008; this study), which is in line with the present mapping results showing that the traits do not share any QTLs in the region on chromosome 2. This suggests that wing length is genetically decoupled and can evolve independently of tarsus length, and (since tarsus length is a body-size-related trait) also to some extent independently of body size. Still, other important trade-offs with respect to migration efficiency that we have not studied may occur, e.g. between heavy flight muscles and wing length. Large flight muscles would be necessary for full usage of a long wing, but at the cost of increasing body mass and thus decreasing flight performance. However, such morphologically different characters are less likely to be controlled by the same functional genes. Wing length is a crucial trait for long-distance migrants (Savile 1956; Alatalo *et al.* 1984*b*; Mulvihill & Chandler 1990) and a fast phenotypic response would be adaptive during rapid northwards range expansions as in our study species (Hansson *et al.* 2008). As mentioned above, there is a pronounced latitudinal gradient in wing length in great reed warblers, with southern populations with short migratory distances having substantially shorter wings (e.g. 95.6 mm in Turkey) than northern populations (e.g. 101.3 mm in Sweden). This pattern strongly suggests that wing length is associated with migration distance over great reed warbler's geographical range, and this issue can now be addressed by trait-specific phenotypic and genetic screens of populations along latitudinal gradients.

In addition to this large-scale selection regime, wing length is also under sexually antagonistic selection within local great reed warbler populations, with females experiencing negative and males positive directional selection (Åkesson *et al.* 2007*b*; Tarka *et al.* in preparation). Further studies of the QTL region in our main great reed warbler study population have the potential to reveal how variance is maintained in traits under selection, unravel the genetic architecture of a trait in intralocus sexual conflict and shed light on evolutionary constraints. A prime challenge for future analyses will be to evaluate to which degree intra- and interpopulation evolutionary processes, respectively, affect the genetic variation in wing length in the great reed warbler. Our QTL mapping efforts have now opened up the possibility to study these processes at the locus level. The fact that we did find a QTL, despite using a low-density linkage map with moderate mapping power, should encourage and trigger QTL analyses in wild populations of other species. This could, in turn, shed new light on the processes maintaining genetic variation of ecologically and evolutionary interesting traits under natural conditions.

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